09/980531

JC10 Rec'd PCT/PTO 26 OCT 2001

Express Mail No.: **EL 865331272 US** Date: **October 26, 2001**

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ANTONELLA FUSILLO
(Name of person mailing paper or fee)

(Signature)

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		Attorney's Docket No: ARDEN-JACOB
INTERNATIONAL APPLICATION NO. PCT/EP00/03568	INTERNATIONAL FILING DATE 19 April 2000	PRIORITY DATE CLAIMED 27 April 1999

TITLE OF INVENTION
NOVEL CARBOPYRONINE FLUORESCENT DYES

APPLICANT(S) FOR DO/EO/US
JUTTA ARDEN-JACOB, JÖRG FRANTZESKOS & ALEXANDER ZILLES

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)

6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [ ] Original or facsimile of an oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [X] Other items or information: International Search Report and Form PTO-1449, Form PCT/IB/308

JC10 Rec'd PCT/PTO 26 OCT 2001

U.S.APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/980531	PCT/EP00/03568	ARDEN-JACOB

17. [X] The following fees are submitted :
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):

[X] For filing with EPO or JPO search report (37 C.F.R. 1.492(a)(5))	$ 890.00	$890.00
[ ] International preliminary examination fee paid to USPTO (37 C.F.R. 1.492(a)(1))	$ 710.00	
[ ] No international preliminary examination fee paid to USPTO (37 C.F.R. 1.492(a)(2)) but international search fee paid to USPTO (37 C.F.R. 1.445(a)(2))	$ 740.00	
[ ] Neither international preliminary examination fee paid to USPTO (37 C.F.R. 1.492(a)(3)) nor international search fee paid to USPTO (37 C.F.R. 1.445(a)(2))	$1,040.00	
[ ] International preliminary examination fee paid to USPTO (37 C.F.R. 1.492(a)(4)) and all claims satisfied provisions of PCT Articles 33(2)-33(4)	$ 100.00	

Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

Claims	Number Field	Rate	
Total Claims	19-20	x $ 18.00	
Independent Claims	1-3	x $ 84.00	
Multiple dependent claims (if applicable)		x $280.00	
		TOTAL OF ABOVE CALCULATIONS	$890.00
[X] Applicant claims small entity status pursuant to 37 C.F.R. 1.27. Reduction by 1/2 for filing by small entity.			
		SUBTOTAL	$445.00
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date 37 CFR 1.492(f).			
		TOTAL NATIONAL FEE	$445.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +			$ 0.00
		TOTAL FEES ENCLOSED	$445.00
		Amount to be refunded	
		charged	

a. [X] A check in the amount of **$445.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **06-0502** in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **06-0502**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

Send all correspondence to:

HENRY M. FEIEREISEN
350 Fifth Avenue
Suite 3220
New York, N.Y. 10118
(212) 244-5500
Date: October 26, 2001

HENRY M. FEIEREISEN
Registration No. 31,084

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No.: ARDEN-JACOB

In re Application of:	)
JUTTA ARDEN-JACOB et al.	)
Int. Appl. No.: PCT/EP00/03568	)
Int. Filing Date: April 19, 2000	)
For: NOVEL CARBOPYRONINE FLUORESCENT DYES	)

FIRST PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Express Mail mailing label number: **EL 865331272 US**
Date of Deposit: **October 26, 2001**
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

ANTONELLA FUSILLO
(Name of person mailing paper or fee)

(Signature)

S I R:

Preliminary to the first Official Action in the above-entitled application, please amend the application as follows.

The Commissioner is hereby also authorized to charge any fees which may be required during the pendency of this application, or credit any overpayment to Deposit Account No: 06-0502: any patent application processing fees under 37 C.F.R. 1.17, and any filing fees under 37 C.F.R. 1.16, including presentation of extra claims.

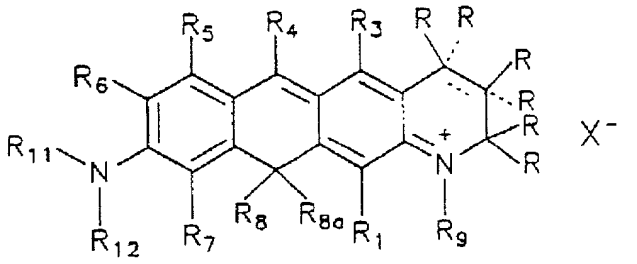
CLEAN VERSION OF AMENDED CLAIMS:

3. The use as claimed in claim 1, characterized in that the detection procedure is selected from nucleic acid hybridization procedures and immunochemical procedures.

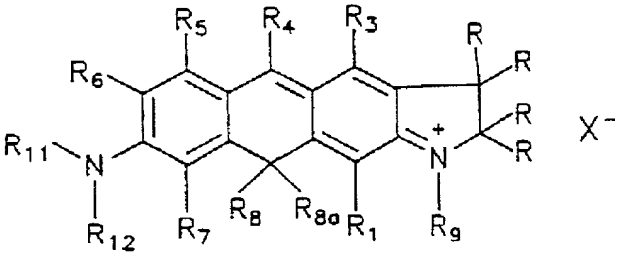
6. (Amended) A compound as claimed in claim 4, characterized in that R_4 is hydrogen, C_1-C_6-alkyl or a radical containing an aromatic ring system.

7. (Amended) A compound as claimed in claim 4, characterized in that R_8 and R_{8a} are in each case independently methyl, ethyl or/and phenyl.

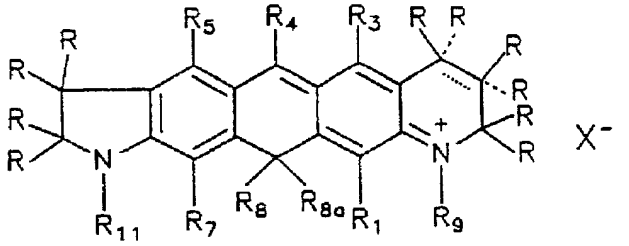
8. (Amended) A compound as claimed in claim 4, which corresponds to one of the general formulae IVa to IVe.



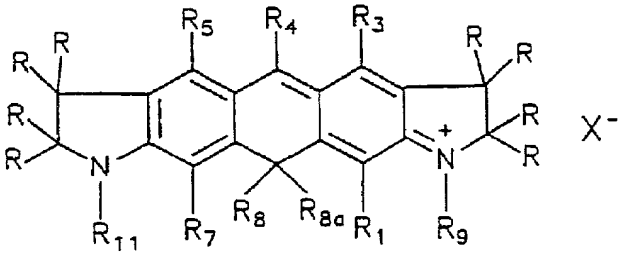
IVa



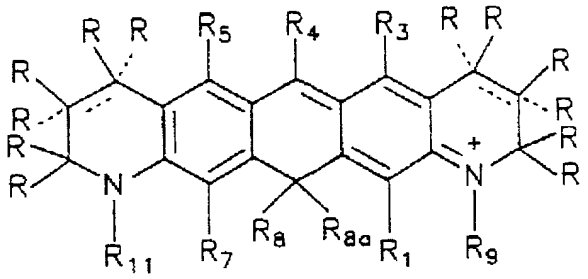
IVb



IVc



IVd



IVe

in which

the dashed lines are optionally double bonds, and in the presence of the double bonds the radicals R bonded via a dashed line are absent,

R_1, R_3, R_4, R_5, R_6, R_7, R_8, R_{8a}, R_9, R_{11}, R_{12} and X are as defined in claim 1,

and R, on each occurrence, can be identical or different and is defined as R_1-R_7 in claim 1.

9. (Amended) A compound as claimed in claim 4, characterized in that it has a group capable of covalent coupling.

11. (Amended) A compound as claimed in claim 9, characterized in that it is coupled to a carrier or/and to a biomolecule via coupling groups.

16. (Amended) The process as claimed in claim 14, characterized in that the catalyst is boron trichloride.

17. (Amended) The process as claimed in claim 14, characterized in that the acid is sulfuric acid, phosphoric acid or polyphosphoric acid.

18. (Amended) The process as claimed in claim 14, characterized in that the oxidant is tetrabutylammonium (meta)periodate.

19. (Amended) The process as claimed in claim 14, characterized in that the compound (I) is obtained without isolation of intermediates.

VERSION WITH MARKINGS TO SHOW CHANGES MADE:

IN THE SPECIFICATION:

Page 1, before line 5, change "Description" to --BACKGROUND OF THE INVENTION--.

Page 2, before line 3, add the heading --SUMMARY OF THE INVENTION--.

Page 33, after the heading "CLAIMS" and before the first claim add --What is claimed is:--.

IN THE CLAIMS:

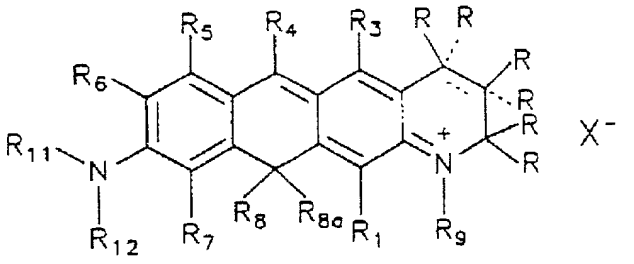
Amend the following claims:

3. The use as claimed in claim 1 ~~or 2~~, characterized in that the detection procedure is selected from nucleic acid hybridization procedures and immunochemical procedures.

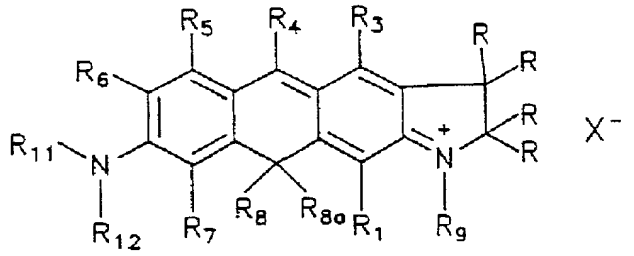
6. (Amended) A compound as claimed in claim 4 ~~or 5~~, characterized in that R_4 is hydrogen, C_1-C_6-alkyl or a radical containing an aromatic ring system.

7. (Amended) A compound as claimed in ~~one of claims~~ claim 4 ~~to 6~~, characterized in that R_8 and R_{8a} are in each case independently methyl, ethyl or/and phenyl.

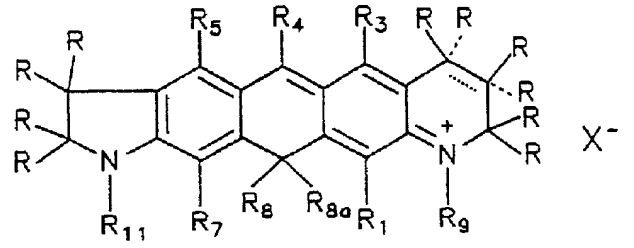
8. (Amended) A compound as claimed in ~~one of claims~~ claim 4 ~~to 6~~, which corresponds to one of the general formulae IVa to IVe.



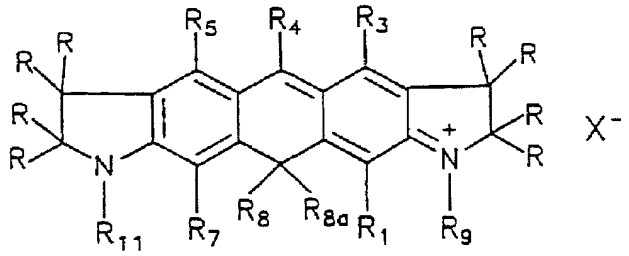
IVa



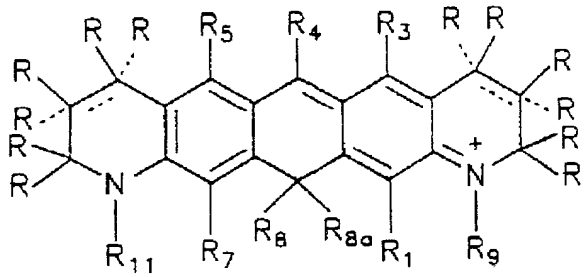
IVb



IVc



IVd



IVe

in which

the dashed lines are optionally double bonds, and in the presence of the double bonds the radicals R bonded via a dashed line are absent,

R_1, R_3, R_4, R_5, R_6, R_7, R_8, R_{8a}, R_9, R_{11}, R_{12} and X are as defined in claim 1,

and R, on each occurrence, can be identical or different and is defined as R_1-R_7 in claim 1.

9. (Amended) A compound as claimed in ~~one of claims~~ claim 4 ~~to 8~~, characterized in that it has a group capable of covalent coupling.

11. (Amended) A compound as claimed in claim 9 ~~or 10~~, characterized in that it is coupled to a carrier or/and to a biomolecule via coupling groups.

16. (Amended) The process as claimed in ~~one of claims~~ claim 14 ~~to 15~~, characterized in that the catalyst is boron trichloride.

17. (Amended) The process as claimed in ~~one of claims~~ claim 14 ~~to 16~~, characterized in that the acid is sulfuric acid, phosphoric acid or polyphosphoric acid.

18. (Amended) The process as claimed in ~~one of claims~~ claim 14 ~~to 17~~, characterized in that the oxidant is tetrabutylammonium (meta)periodate.

19. (Amended) The process as claimed in ~~one of claims~~ claim 14 ~~to 18~~, characterized in that the compound (I) is obtained without isolation of intermediates.

REMARKS

This Amendment is submitted preliminary to the issuance of an Office Action in the present application.

Applicant has amended claims 3, 6-9, 11, 16-19 to remove any multiple dependency of the claims. In addition, applicant has amended the specification to present it with proper headings.

When the Examiner takes this application up for action, it is requested to take the foregoing into account.

The Commissioner is hereby authorized to charge fees which may be required, or credit any overpayment to Deposit Account No. 06-0502.

Respectfully submitted,

By: _____
Henry M. Feiereisen
Agent for Applicant
Reg. No. 31,084

Date: October 26, 2001
350 Fifth Avenue
Suite 3220
New York, N.Y. 10118
(212) 244-5500
HMF:af

Novel carbopyronine fluorescent dyes

Description

The invention relates to the use of carbopyronine compounds of the general formula (I) as labeling groups in procedures for the detection of analytes, to novel carbopyronine compounds and to a process for the preparation of these compounds.

In chemical, medical and biological analysis, dyes are used as labeling or detection groups. In particular, fluorescent dyes have gained importance in recent years and displaced other often cost-intensive procedures, which use, for example, radioisotopes for labeling.

In particular in the field of DNA sequencing, fluorometric procedures have gained acceptance in recent years and almost completely replaced the procedures customary up till then, which use radioactive isotopes.

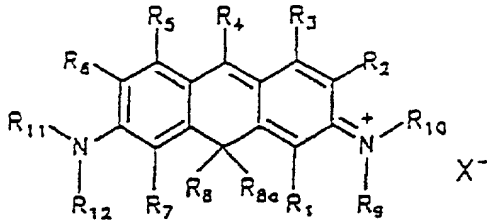
In spite of the availability of various fluorescent dyes, such as, for example, FITC (fluorescein isothiocyanate), FLUOS (fluorescein N-hydroxy-succinimide ester), rhodamine derivatives etc., it was previously not possible to solve the problems due to background fluorescence, nonspecific binding phenomena and the need for cost-intensive measuring equipment in a satisfactory manner.

As a result of background fluorescence and nonspecific binding, the sensitivity and accuracy of the measurements is reduced. In addition, in the case of available fluorescent dyes the absorption maximum lies in regions which do not make possible the use of light sources which are less expensive and which can be of small dimensions, such as, for example, He/Ne lasers

and laser diodes.

An object of the present invention was thus to make available fluorescent dyes which can be employed as labeling groups in procedures for the detection of analytes and at least partially avoid the disadvantages of the prior art.

This object has been achieved by the use of compounds of the general formula (I)



I

as labeling groups in a procedure for the detection of an analyte, where

R_1, R_2, R_3, R_4, R_5, R_6 and R_7 are in each case independently hydrogen, halogen, a hydroxyl, amino, sulfo or carboxyl or aldehyde group or a saturated or unsaturated, straight-chain, branched or cyclic hydrocarbon group having up to 20 C atoms, where the hydrocarbon groups include alkyl, alkenyl, alkynyl, cycloalkyl, aryl, in particular phenyl, or/and heteroaryl radicals and optionally heteroatoms such as oxygen, sulfur or nitrogen atoms or/and two or more substituents, preferably selected from halogens, hydroxyl, amino, sulfo, phospho, carboxyl, aldehyde, C_1-C_4-alkoxy or/and C_1-C_4-alkoxycarbonyl groups, or one or more of the radicals R_1-R_7, in each case with adjacent substituents, form a ring system which can contain one or more multiple bonds,

R_8 and R_{8a} in each case independently are a saturated or unsaturated, straight-chain, branched or cyclic hydrocarbon group having up to 20 carbon atoms, e.g. a C_1-C_6-alkyl group, in particular methyl, ethyl, propyl or/and butyl, or an aryl or heteroaryl group, in

particular phenyl, which optionally contain heteroatoms such as oxygen, sulfur or nitrogen atoms or/and one or more substituents, preferably selected from halogens, hydroxyl, amino, sulfo, phospho, carboxyl, aldehyde, C_1-C_4-alkoxy or/and C_1-C_4-alkoxycarbonyl groups, or R_8 and R_{8a} can form a ring system,

R_9, R_{10}, R_{11} and R_{12} in each case independently are hydrogen or a saturated or unsaturated, straight-chain, branched or cyclic hydrocarbon group having up to 20 C atoms, e.g. polyether, phenyl, phenylalkyl having 1-3 C atoms in the chain, where the hydrocarbon groups can optionally contain heteroatoms such as oxygen, sulfur or nitrogen atoms or/and one or more substituents, preferably selected from halogens, hydroxyl, amino, sulfo, phospho, carboxyl, carbonyl, alkoxy or/and alkoxycarbonyl groups,

or one or more of the radicals R_9-R_{12}, in each case with adjacent substituents, form a ring system which can contain one or more multiple bonds,

where $-N(R_9)(R_{10})$ or/and $=N(R_{11})(R_{12})$ can be replaced by $-OR^9$ or/and $=O$,

and X is optionally anions present for charge equalization.

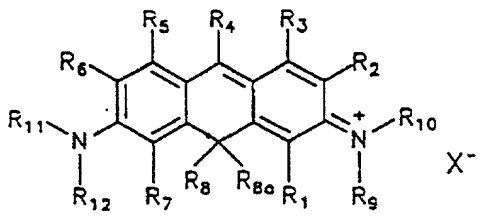
The compounds of the general formula (I) can be employed as labeling groups in procedures for the qualitative or/and quantitative determination of an analyte. This determination can be carried out in aqueous liquids, e.g. samples of body fluids such as, for example, blood, serum, plasma or urine, waste water samples or foodstuffs. The procedure can also be carried out as a wet test, e.g. in a cuvette, or as a dry test in an appropriate reagent carrier. The determination of the analyte can be carried out here by means of a single reaction or by means of a sequence of reactions. Surprisingly, the use of compounds of the general formula (I) showed very good results in chemical and in particular in medical and biological detection procedures for the determination of an

analyte, especially in nucleic acid sequencing procedures and in protein analysis.

The compounds of the general formula (I) can be used in all chemical, medical and biological detection procedures known to the person skilled in the art in which fluorescent dyes are suitable as labeling groups. For this, the compounds of the general formula (I) are in general coupled covalently to a receptor which is specific for the analyte to be detected. This takes place using generally known procedures. The specific receptor can be any suitable compound or any suitable molecule, preferably it is a peptide, a polypeptide or a nucleic acid. The compounds or conjugates of these compounds can be used, for example, in nucleic acid hybridization procedures, in particular for the sequencing of nucleic acids or immunochemical procedures. Procedures of this type are described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual, 1989, Cold Spring Harbor.

A further object of the present invention was to make available novel carbopyronine compounds which are suitable in particular for use as labeling groups in analyte detection procedures, can be prepared using simple and inexpensive processes, can be handled without problems and at least partially avoid the disadvantages of the prior art.

This object has been achieved by a compound of the general formula (I)



I

where R_1-R_{12} and X have the meanings indicated above,

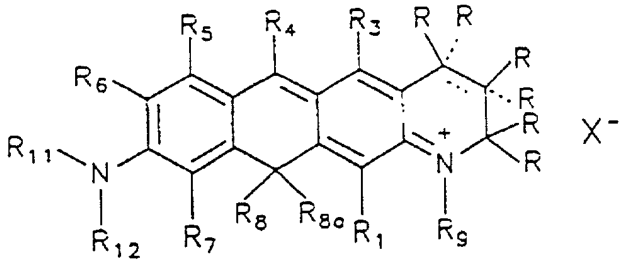
with the proviso that if R_1-R_3 and R_5-R_7 are hydrogen and R_8, R_{8a} and R_9-R_{12} are methyl,
R_4 is not hydrogen, methyl, isopropyl, phenyl, 2,6-dimethylphenyl or 2-isopropenylphenyl.

An advantage of the compounds (I) is that owing to an almost arbitrary substituent variation the properties of individual compounds, e.g. the spectroscopic properties, the position of the absorption and fluorescence maxima, the solubility properties, the fluorescence quantum yield and decay time, vary strongly and thus can be selected as desired. In this way, interferences with interfering substances in samples such as serum, blood or plasma etc. can be reduced or even avoided completely. The preparation of some compounds of the formula (I) can be carried out by processes known per se. Preferably, the synthesis is carried out, however, according to a novel process described below, which is particularly simple and inexpensive.

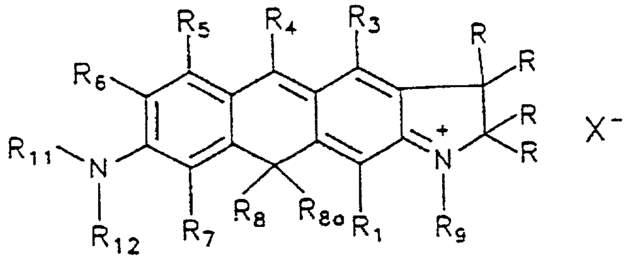
In a preferred class of the compounds (I), R_6 is bridged with R_{11} or/and R_7 with R_{12}, R_1 with R_{10} or/and R_2 with R_9 and form a ring system which can contain one or more multiple bonds. The ring system preferably contains one or more 5- or 6-membered rings.

R_4 is preferably hydrogen, C_1-C_6-alkyl or a radical containing an aromatic ring system, e.g. a radical containing a carboxyl or/and halogen group, such as 2-carboxyphenyl, 2-carboxytetrachlorophenyl or pentafluorophenyl. R_8 and R_{8a} are preferably in each case independently methyl, ethyl or/and optionally substituted phenyl.

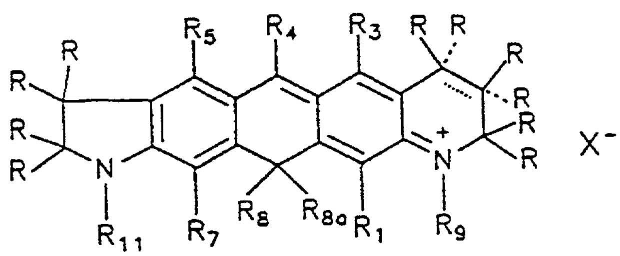
Examples of particularly preferred classes of compound are shown in the general formulae IVa to IVe:



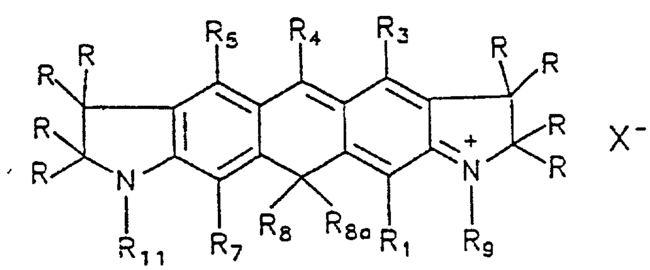
IVa



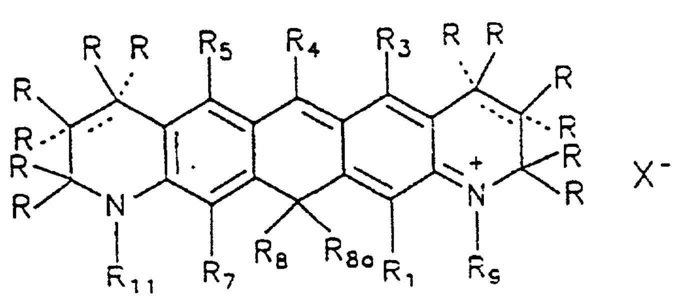
IVb



IVc



IVd



IVe

in which the dashed lines are optionally double bonds, in whose presence the radicals R bonded via a dashed line are absent,

R_1, R_3, R_4, R_5, R_6, R_7, R_8, R_{8a}, R_9, R_{11}, R_{12} and X are as defined above, and R, on each occurrence, can be identical or different and is defined as R_1-R_7 above.

The compounds preferably have a group capable of covalent coupling, e.g. -COOH, $-NH_2$, -OH or/and -SH. By means of this coupling group, the compound can be coupled to a carrier or/and to a biomolecule according to known methods. The carrier can consist of any material which is suitable, in particular for detection procedures, e.g. of porous glass, plastics, ion-exchange resins, dextrans, cellulose, cellulose derivatives or/and hydrophilic polymers. The biomolecules are preferably selected from peptides, polypeptides, nucleotides, nucleosides, nucleic acids, nucleic acid analogs or/and haptens.

Surprisingly, the absorption maxima and the fluorescence quantum yield are not significantly changed by coupling of the compounds according to the invention to the abovementioned carriers and biomolecules.

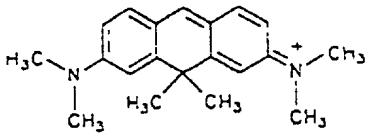
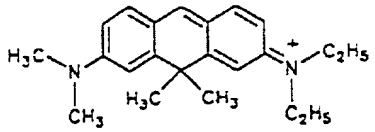
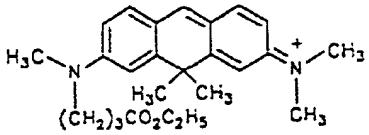
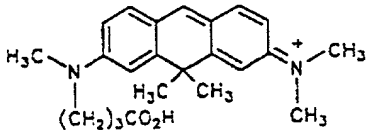
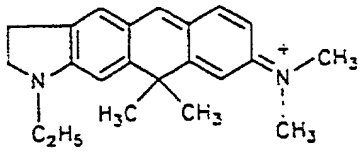
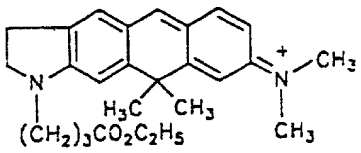
Actual examples of compounds according to the invention are shown in table 1 below.

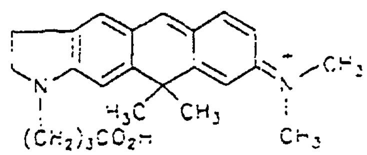
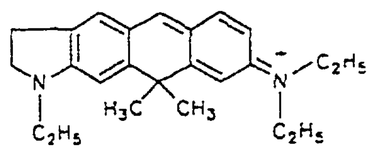
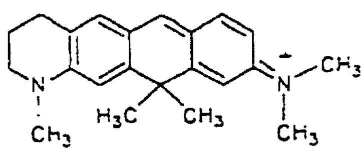
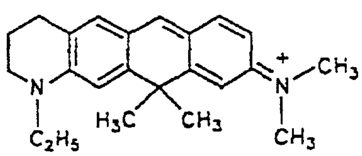
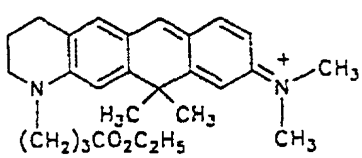
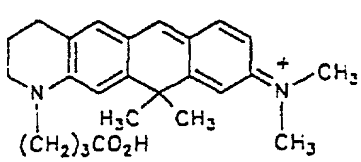
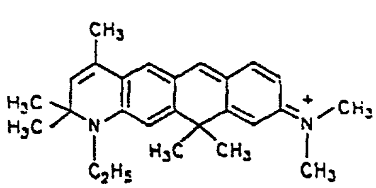
Table 1

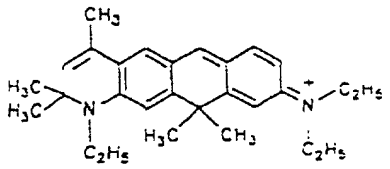
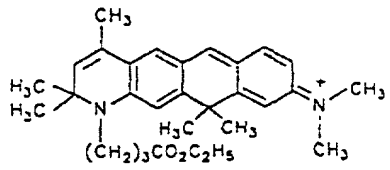
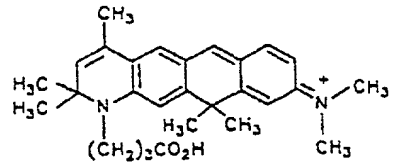
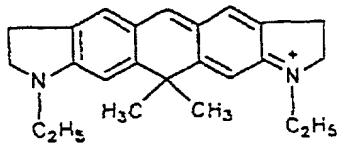
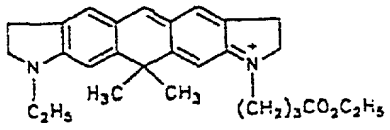
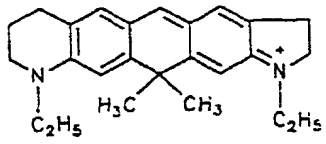
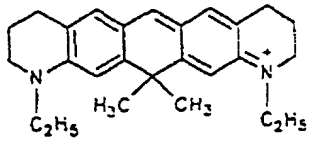
λ_A: absorption maximum

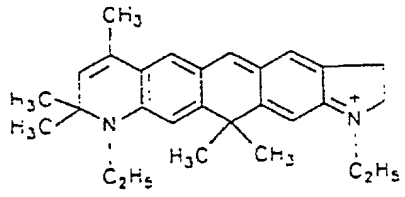
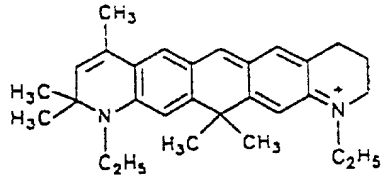
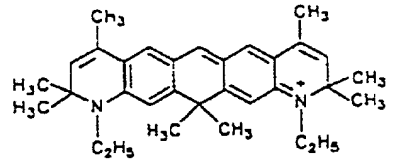
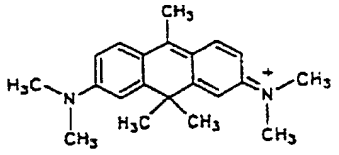
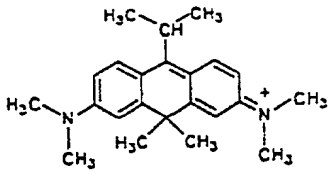
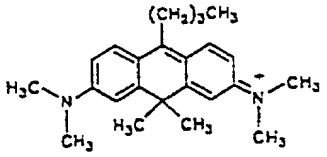
λ_F: fluorescence maximum

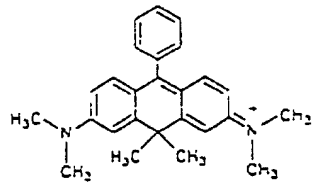
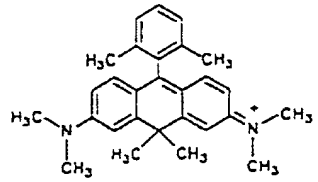
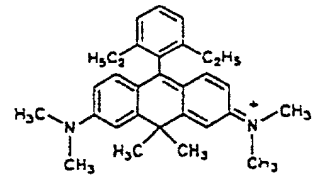
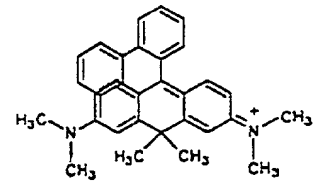
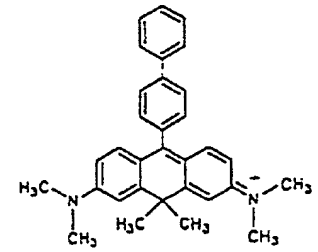
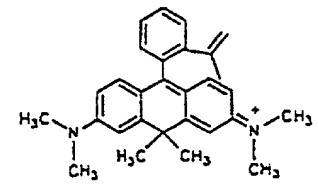
Q_F: fluorescence quantum yield in ethanol

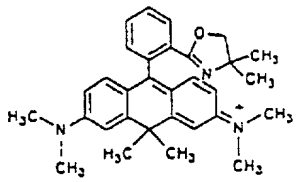
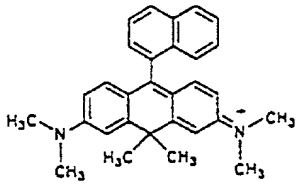
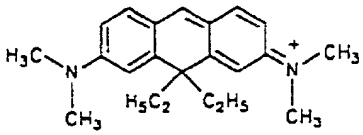
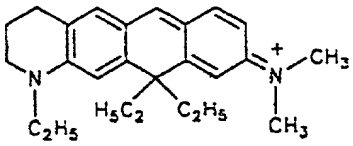
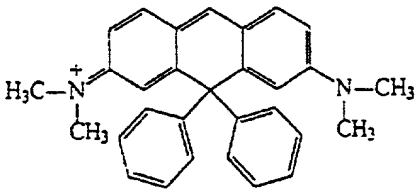
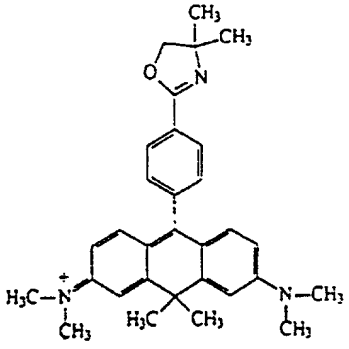
	Structure	λ_A / nm	λ_F / nm	Q_F / %
1 Cp 149		606	627	71
2 AZ 6		608	630	65
3 JA 261		608	630	70
4 JA 262		608	630	70
5 AZ 1		617	641	77
6 AZ 4		617	641	78

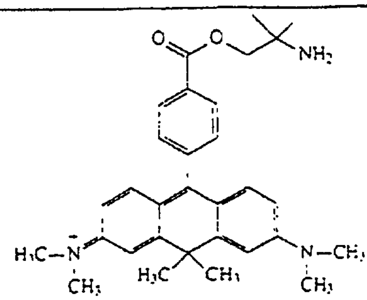
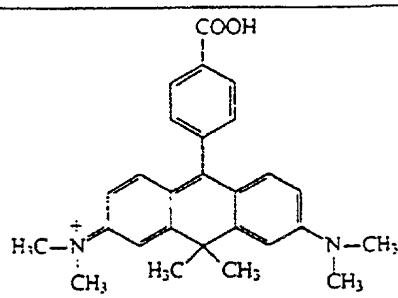
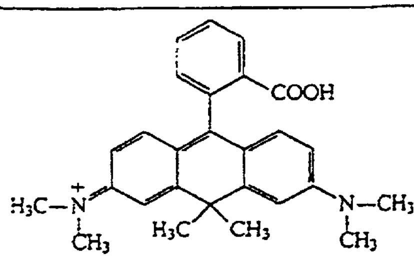
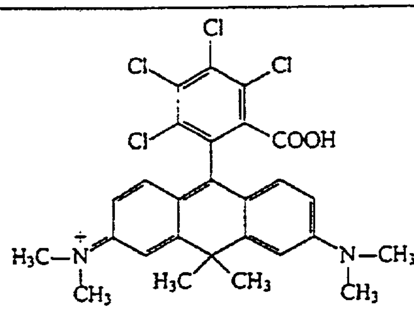
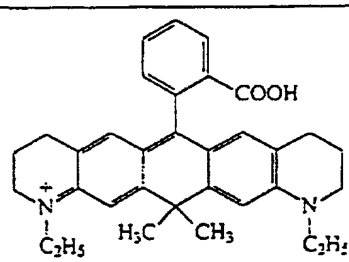
7 AZ 14		617	641	78
8 AZ 7		618	642	75
9 JA 260		616	640	75
10 JA 264		616	640	75
11 JA 263		616	640	76
12 JA 266		616	640	76
13 JA 265		634	658	62

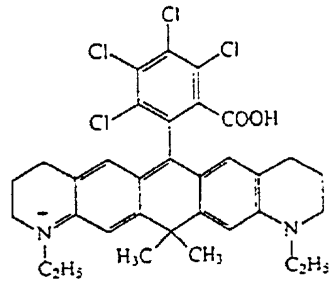
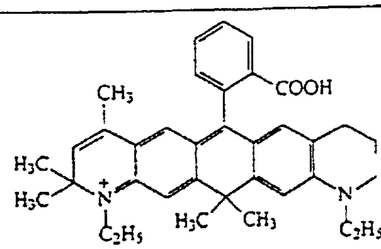
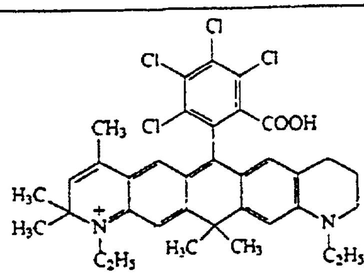
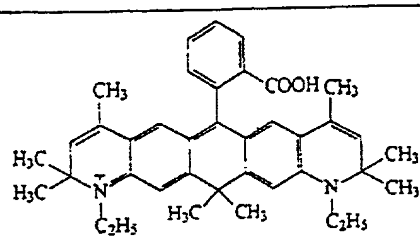
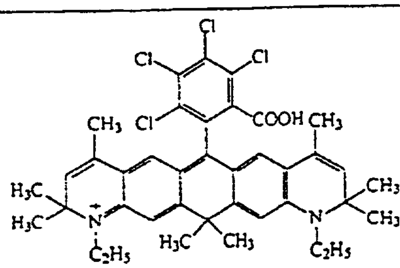
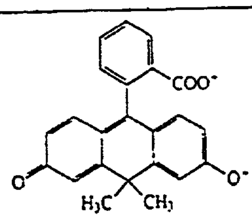
14 AZ 8		641	666	60
15 JA 267		633	660	60
16 JA 268		634	660	58
17 AZ 2		633	657	63
18 AZ 5		633	657	61
19 AZ 3		629	650	69
20 AZ 13		626	648	87

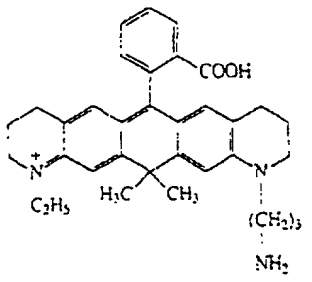
21 AZ 9		647	675	55
22 AZ 12		647	664	58
23 AZ 11		664	688	49
24 JF 19		602	643	58
25 JF 20		604	675	41
26 JF 18		601	636	67

27 JF 16		611	638	6
28 JF 21		610	637	46
29 JF 22		612	641	41
30 JF 24		617	643	71
31 JF 25		613	638	6
32 JF 26		611	640	59

33 JF 17		610	640	70
34 JF 23		618	643	60
35 AZ 16		606	628	70
36 AZ 17		615	640	75
37 AZ 18		627	655	62
38 JF30		621	652	4

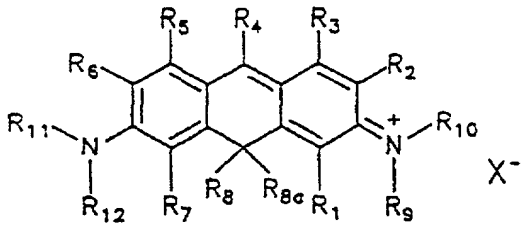
39 JF 31		618	648	5
40 JF 32		618	647	5
41 JF 34		612	642	75
42 JF 35		642	672	64
43 JF 36		632	662	85

44 JF 37		662	692	60
45 JF 38		653	683	70
46 JF 39		683	713	45
47 JF 40		670	700	55
48 JF 41		700	730	40
49 JF 42		557	577	95

50 JF 43		632	660	80

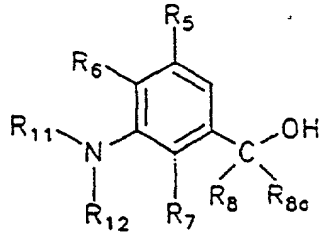
A further object of the present invention consisted in making available a preparation process for carbopyronine compounds which can be carried out in a simple, environmentally compatible and inexpensive manner and which at least partially avoids the disadvantages of the known processes for the preparation of carbopyronines.

This object was achieved according to the invention by a process for the preparation of compounds of the general formula (I)



I

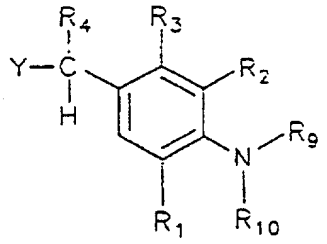
where R_1-R_{12} and X have the meanings indicated in claim 1, characterized in that a compound of the general formula (II)



II

in which R_5, R_6, R_7, R_8, R_{8a}, R_{11} and R_{12} are as defined

above, or the dehydration product of II is reacted with a compound of the general formula III or its dehydration product



III

in which R_1-R_4, R_9 and R_{10} are as defined above and Y is a halogen, in particular bromine, a hydroxyl or thio group,

in a suitable solvent, under acidic conditions and in the presence of a catalyst and the compound formed by ring closure between the compounds II or their dehydration product and III are reacted by oxidation to give the structure I. Alternatively, the compounds II or their dehydration product can be reacted with the dehydration product of III, the structure I being formed directly - without an oxidation step.

In the process, it is possible to use all suitable solvents which are compatible with the starting materials, the products and the catalyst, preferably boron trichloride. The solvent is preferably a nonpolar solvent, in particular methylene chloride, 1,2-dichloroethane or chloroform.

The acids employed can be customary acids. The acid is preferably an inorganic acid such as sulfuric acid, phosphoric acid or polyphosphoric acid.

The oxidants used can likewise be customary oxidants. The oxidant tetrabutylammonium (meta)periodate is preferred.

It is particularly advantageous that the process can be

carried out without isolation of intermediates. This leads to a reduction in the expenditure of time, labor and material.

The invention is illustrated in greater detail by the examples below. The figures 1, 2, 3 and 4 show the absorption and fluorescence spectra of the compounds according to the invention AZ 2 (17), AZ 13 (20), JA 268 (16) and AZ11 (23).

Examples

A. Preparation process according to the invention for carbopyronine compounds

In the process according to the invention, 4-N,N-dimethylaminobenzylsulfanilic acid, which is used in the process according to Aaron and Barker (J. Chem. Soc. (1963), 2655) is replaced by 4-hydroxymethyl-N,N-dimethylaniline and reacted with the isopropenyl derivative to give the carbopyronine in the presence of boron trichloride solution as a catalyst. The reaction mixture can be reacted with concentrated sulfuric acid to give the leuko base of the dye without isolation of the intermediate. The oxidant lead dioxide used in Aaron and Barker (loc. cit.) is replaced by tetrabutylammonium (meta)periodate. To this end, the ethanolic solution of oxidant and leuko base is heated to boiling, it being possible to detect by thin-layer chromatography that the oxidation is already complete after a few minutes.

After the oxidation, the carbopyronine is precipitated from ethanolic solution as a poorly soluble perchlorate by addition of 10% strength sodium perchlorate solution and slow dropwise addition of water.

The novel synthesis route can be employed universally. The corresponding alcohols can be obtained from

aniline, indoline, tetrahydroquinoline and 1,2-dihydroquinoline derivatives by a Vilsmaier synthesis with subsequent reduction and these can be reacted with an isopropenyl derivative to give the dye. Unlike the synthesis of Aaron and Barker, the synthesis proceeds in one step, i.e. isolation of intermediates is not necessary.

The synthesis procedures for the compounds JA 261, JA 262, AZ 4, AZ 14, JA 267, JA 268, JF 19, JF 22 and JF 17 are presented below.

B. Synthesis examples

Compound JA 261

1 g (4 mmol) of ethyl N-methyl-N-(4-hydroxymethylphenyl)-4-aminobutyrate and 0.71 g (4.4 mmol) of 3-(isopropenyl)-N,N-dimethylaniline are dissolved in 20 ml of methylene chloride. 4 ml of a 1 molar BCl_3 solution (in methylene chloride) are slowly added with stirring and ice cooling. The solution is stirred overnight at room temperature. The reaction mixture is then added dropwise to 20 g of concentrated sulfuric acid, which is cooled in an ice/methanol bath. The mixture is stirred until a homogeneous solution is present. The methylene chloride is distilled off on a rotary evaporator. The sulfuric acid solution is stored overnight in a refrigerator. The solution is then poured onto ice and neutralized with dilute sodium hydroxide solution. The aqueous solution is extracted with chloroform. The combined organic phases are dried over sodium sulfate, filtered and concentrated to dryness on a rotary evaporator. The residue is taken up in 200 ml of ethanol and treated with 10 drops of 60% strength perchloric acid and 0.17 g (0.39 mmol) of tetrabutylammonium (meta)periodate. The solution is heated to reflux for 30 min. The cooled solution is added dropwise to a solution of 20 g of sodium perchlorate in 1 l of water. The mixture is stirred

overnight. The green, lustrous precipitate is filtered off and dried over phosphorus pentoxide in a desiccator.

Yield: 0.56 g

^{1}H-NMR data in $CDCl_3$:

δ 1.25 (T, 3H, $-CH_3$); 1.7 (S, 6H, $-CH_3$); 2.0 (QI, 2H, $-CH_2-$); 2.5 (T, 2H, $-CH_2-$); 3.3 (S, 9H, $N-CH_3$); 3.7 (T, 2, $-CH_2-$); 4.15 (Q, 2H, $N-CH_2-$); 6.85 (DvD, 2H, ArH); 7.05 (D, 1H, ArH); 7.2 (D, 1H, ArH); 7.65 (D, 2H, Ar-H); 8.0 (S, 1H, -CH=)

Compound JA 262

100 mg of JA 261 are dissolved in a mixture of 20 ml of acetone, 40 ml of water and 2 ml of 2 N hydrochloric acid. The solution is heated to reflux (internal temperature: 64°C). After 24 h, the solution is cooled and treated with 100 ml of 10% strength aqueous sodium perchlorate solution. The precipitate is filtered off and dried.

Yield: 0.04 g.

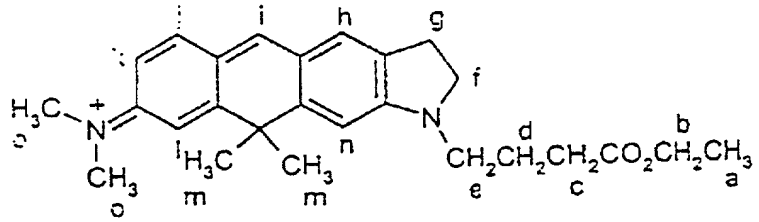
Compound AZ 4

1.00 g (4.25 mmol) of ethyl 4-(5-hydroxymethylindolin-1-yl)butyrate and 0.76 g (4.25 mmol) of 3-(isopropenyl)-N,N-dimethylaniline are dissolved in 15 ml of methylene chloride and treated dropwise with 4.25 ml (4.25 mmol) of a 1 molar solution of boron trichloride in hexane with ice cooling. The reaction mixture is stirred at room temperature for 30 min. The reaction mixture is then added dropwise to 10 ml of concentrated sulfuric acid and stirred at room temperature for 1 h. The deep red-colored reaction mixture is added dropwise to 100 ml of ice-cold ethanol, treated with 0.78 g (1.8 mmol) of tetrabutylammonium (meta)periodate and heated to boiling for 3 min. It is allowed to cool to room temperature and is treated with 50 ml of 20% strength sodium perchlorate solution. 300 ml of water

are then added dropwise to precipitate the dye completely. The crystalline product is filtered off and dried in vacuo in a desiccator using SICAPENT®.

Yield: 0.7 g

^{1}H-NMR data in acetone-d_6:

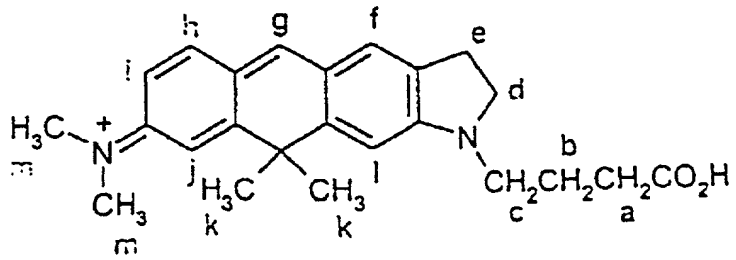


δ 0.9 (T, 3H, $-CH_3$ a); 1.7 (S, 6H, $-CH_3$ m); 2.47 (T, 2H, $-CH_2$-c); 3.22 (T, 2H, $-CH_2$- g); 3.34 (S, 6H, N-CH_3 o); 3.8 (T, 2, $-CH_2$- e); 4.09 (T, 2H, $-CH_2$- f); 4.42 (Q, 2H, $-CH_2$- b); 6.95 (DvD, 1H, ArH k); 7.22 (D, 1H, ArH l); 7.3 (S, 1H, ArH n); 7.7 (D, 1H, Ar-H j); 8.08 (S, 1H, -CH= i)

Compound AZ 14

4 g (8 mmol) of AZ 4 are dissolved in 30 ml of water and 20 ml of acetone and treated with 1 ml of 2 N hydrochloric acid. The reaction mixture is heated to reflux for 18 h. It is treated with 50 ml of chloroform and the organic phase is separated off. After extraction with chloroform a further three times, the combined organic phases are washed with water and dried over sodium sulfate. The dye solution is concentrated to dryness on a rotary evaporator and then purified by column chromatography.

^{1}H-NMR data in acetone-d_6:



δ 1.72 (S, 6H, $-CH_3$ k); 2.0 (M, 2H, $-CH_2-$ b); 2.49 (T, 3H, $-CH_2-$ a); 3.25 (T, 2H, $-CH_2-$ e); 3.34 (S, 6H, $-CH_3$ m); 3.81 (T, 2, $-CH_2-$ c); 4.11 (T, 2H, $-CH_2-$ d); 6.95 (DvD, 1H, ArH i); 7.22 (D, 1H, ArH j); 7.3 (S, 1H, ArH l); 7.42 (S, 1H, Ar-H f); 7.7 (D, 1H, ArH h); 8.1 (S, 1H, -CH= g)

Compound JA 267

1.2 g (3.8 mmol) of ethyl 4-(6-hydroxymethyl-2,2,4-trimethyl-1,2-dihydroquinol-1-yl)butyrate and 0.68 g (3.8 mmol) of 3-(isopropenyl)-N,N-dimethylaniline are dissolved in 30 ml of methylene chloride. 4 ml of a 1 molar BCl_3 solution in methylene chloride are added slowly with stirring and ice cooling. The solution is stirred at room temperature for 20 min. The reaction mixture is then added dropwise to 20 ml of conc. sulfuric acid. It is stirred until a homogeneous solution is present. The methylene chloride is distilled off on a rotary evaporator and the sulfuric acid solution is stirred at room temperature for 1 h. The residue is taken up in 400 ml of ice-cooled ethanol. 1.2 g (2.7 mmol) of tetrabutylammonium (meta)periodate are added thereto. The solution is briefly heated to boiling, cooled and treated with 200 ml of a 20% strength sodium perchlorate solution. 500 ml of water are then added dropwise. The precipitate is filtered off and dried in a desiccator.

Compound JA 268

1.8 g of JA 267 are heated to reflux for 6 h in a mixture of 50 ml of acetone, 50 ml of water and 5 ml of 2 N hydrochloric acid. The solvent is distilled off and the residue is purified by chromatography.

Compound JF 19

0.27 ml (0.81 mmol) of a 3 M methylmagnesium bromide solution in diethyl ether are added dropwise within an argon protective gas atmosphere at room temperature to a solution of 50 mg (0.16 mmol) of 2,10-bis(dimethylamino)anthrone in 10 ml of dry tetrahydrofuran. After reaction is complete, the reaction mixture is cooled in an ice-water bath, dissolved in 50 ml of ethanol and acidified with trifluoroacetic acid. This solution is suspended in a mixture of 50 ml of chloroform and 50 ml of water. The organic phase is separated off, concentrated to dryness on a rotary evaporator and dissolved in ethanol. The solution is then added dropwise to 100 ml of aqueous 25% strength sodium perchlorate solution. After addition is complete, a further 300 ml of water are added dropwise. The dye precipitated is filtered and dried in vacuo.
Yield: 0.04 g

Compound JF 22

Under protection by argon, 11 mg (1.6 mmol) of lithium powder (0.5% sodium, Metallgesellschaft) are suspended in 2 ml of dry diethyl ether. A solution of 0.17 g (0.8 mmol) of 1-bromo-2,6-diethylbenzene in 4 ml of diethyl ether is added dropwise to this suspension with stirring. After addition is complete, the mixture is stirred at room temperature for 15 min. The suspension is filtered through glass wool in order to remove the remaining residues of lithium. The solution thus obtained is added dropwise at room temperature to a solution of 50 mg (0.16 mmol) of 2,10-bis(dimethylamino)anthrone in 10 ml of dry tetrahydrofuran. After reaction is complete, the reaction mixture is cooled in an ice-water bath, dissolved in 50 ml of ethanol and acidified with trifluoroacetic acid. This solution is suspended in a mixture of 50 ml of chloroform and 50 ml of water. The organic phase is separated off, concentrated to dryness on a rotary evaporator and purified by column chromatography on silica gel. After the dye

fraction has been concentrated to dryness on a rotary evaporator, it is dissolved in ethanol and then added dropwise to 100 ml of aqueous 25% strength sodium perchlorate solution. A further 300 ml of water are then added dropwise. The dye precipitated is filtered and dried in vacuo.
Yield: 0.02 g

Compound JF 17

0.14 g (0.55 mol) of 2-(2-bromophenyl)-4,4-dimethyl-2-oxazoline is dissolved in 7.5 ml of tetrahydrofuran under protective gas (argon) and cooled to -78°C. 0.7 ml (1.1 mmol) of a 1.6 M solution of t-butyllithium in hexane are added dropwise to this solution such that the temperature remains below -75°C. After addition is complete, the solution is stirred for 15 min. 34 mg (0.11 mmol) of 2,10-bis(dimethylamino)anthrone in 2 ml of dry tetrahydrofuran are added to this solution. The temperature should not exceed -70°C in the course of this. The mixture is then warmed to -60°C and stirred for 3 h. The cooling bath is removed and the mixture is allowed to warm to room temperature. After 24 h, the reaction mixture is cooled in an ice-water bath, dissolved in 50 ml of ethanol and acidified with trifluoroacetic acid. This solution is suspended in a mixture of 50 ml of chloroform and 50 ml of water. The organic phase is separated off, concentrated to dryness on a rotary evaporator and purified by column chromatography. The dye fraction is concentrated to dryness on a rotary evaporator, taken up in ethanol and subsequently added dropwise to 100 ml of aqueous 25% strength sodium perchlorate solution. After addition is complete, a further 300 ml of water are added dropwise. The dye precipitated is filtered and dried in vacuo.

Compound AZ 18

1st stage:
3-(N,N-Dimethylamino)triphenylcarbinol

2.8 g (0.12 mol) of magnesium and 10 ml of diethyl ether (absolute) are treated with 2.6 g (0.02 mol) of bromobenzene. In order to start the reaction, the mixture is slightly warmed. The start of the reaction can be detected by the turbidity of the reaction mixture. 16.2 g (0.1 mol) of bromobenzene are then dissolved in 15 ml of ether and added dropwise to the reaction mixture. It is heated to reflux for 1 h, the magnesium almost completely dissolving. After cooling in an ice bath, a solution of 10 g (0.055 mol) of methyl 3-dimethylaminobenzoate in 15 ml of absolute ether is added dropwise. After the addition, the reaction mixture is heated to reflux for 2 h, cooled and hydrolyzed dropwise with water. 50 ml of water and 50 ml of ether are added and the mixture is treated with saturated ammonium chloride solution until the white precipitate has dissolved again. The aqueous phase is extracted with ether. The combined organic phases are washed with saturated sodium hydrogencarbonate solution and with water. The solution is then dried over sodium sulfate and the solvent is distilled off. The residual pale yellow oil can be used directly for the subsequent reaction.

2nd stage:
AZ 18

0.6 g (3 mmol) of N,N-dimethyl-4-hydroxymethylaniline and 0.9 g (3 mmol) of 3-(N,N-dimethylamino)triphenylcarbinol are dissolved in 30 ml of methylene chloride. 4 ml of a 1 molar BCl_3 solution in methylene chloride are slowly added with stirring and ice cooling. The solution is stirred at room temperature for 2 h. The reaction mixture is then added dropwise to 20 ml of 70%

strength sulfuric acid. The methylene chloride is distilled off on a rotary evaporator and the sulfuric acid solution is stirred at room temperature for 20 h. The residue is slowly dissolved in 100 ml of ice-cooled ethanol. 1.2 g (2.7 mmol) of tetrabutylammonium (meta)-periodate are added thereto. The solution is briefly heated to boiling, cooled and treated with 100 ml of a 20% strength sodium perchlorate solution. 250 ml of water are then added dropwise. The precipitate is filtered off and dried in a desiccator.

Compound JF 30

1.85 ml (3.05 mmol) of a 15% strength t-butyllithium solution (in n-pentane) are added at -78°C to a solution of 0.39 g (1.53 mmol) of 2-(4-bromophenyl)-4,4-dimethyl-2-oxazoline in 20 ml of dry tetrahydrofuran such that the temperature remains below -70°C. After complete addition, 150 mg (0.48 mmol) of 3,6-bis-(dimethylamino)anthrone in 30 ml of dry tetrahydrofuran are added such that the temperature remains below -60°C. The solution is allowed to warm to room temperature and is stirred at room temperature for 18 h. The reaction mixture is cooled in an ice-water bath, dissolved in 50 ml of ethanol and acidified with trifluoroacetic acid. This solution is suspended in a mixture of 50 ml of chloroform and 50 ml of water. The organic phase is separated off, concentrated to dryness on a rotary evaporator, and purified by column chromatography on silica gel. The dye is eluted using 15% strength ethanolic chloroform. After the product phase has been concentrated to dryness on a rotary evaporator, it is dissolved in ethanol and then added dropwise to 100 ml of aqueous 25% strength sodium perchlorate solution. After addition is complete, a further 300 ml of water are added dropwise. The dye precipitated is filtered and dried over phosphorus pentoxide in a vacuum desiccator.

Yield: 50% (cryst. substance after chromatography)

Compound JF 31

80 mg (0.14 mmol) of JF 30 are heated under reflux for 40 min in 10 ml of a 1:3 mixture of 2 M hydrochloric acid and acetone. The mixture is allowed to cool and is suspended in 50 ml of a 1:1 mixture of chloroform and water. The water phase is neutralized with saturated sodium hydrogencarbonate solution. The organic phase is separated off and the aqueous is extracted a number of times with 20% strength ethanolic chloroform. The combined organic phases are concentrated on a rotary evaporator and purified by column chromatography on silica gel. The dye is eluted using 20% strength ethanolic chloroform. After the product phase has been concentrated to dryness on a rotary evaporator, it is dissolved in ethanol and then added dropwise to 100 ml of aqueous 25% strength sodium perchlorate solution. After addition is complete, a further 300 ml of water are added dropwise. The dye precipitated is filtered and dried over phosphorus pentoxide in a vacuum desiccator.
Yield: 72% (cryst. substance after chromatography)

Compound JF 32

70 mg (0.12 mmol) of JF 31 are heated to reflux for 1 h in a 10% strength sodium hydroxide solution in 1:1 ethanol and water. The mixture is allowed to cool and is suspended in a 1:1 mixture of chloroform and water. It is adjusted to pH = 8 using trifluoroacetic acid and the organic phase is separated off. The aqueous phase is extracted a number of times with 20% strength ethanolic chloroform. This extraction is repeated until there is barely still dye in the aqueous phase (testing by means of acidification). The combined organic phases are adjusted to pH = 2 using trifluoroacetic acid, concentrated on a rotary evaporator and purified by column chromatography on silica gel. The dye is eluted

using 10% strength ethanolic chloroform. After the product phase has been concentrated to dryness on a rotary evaporator, it is dissolved in ethanol and then added dropwise to 100 ml of aqueous 25% strength sodium perchlorate solution. After addition is complete, a further 300 ml of water are added dropwise. The dye precipitated is filtered and dried over phosphorus pentoxide in a vacuum desiccator.
Yield: 57% (cryst. substance after chromatography)

Compound JF 42

70 mg (0.12 mmol) of JF 17 are heated to reflux for 1 h in 30 ml of a solution of 3 g of sodium hydroxide in ethanol/water (1:1). The solution is allowed to cool and is neutralized using semiconcentrated hydrochloric acid. The dye is then precipitated by dropwise addition of water. The product is filtered off and dried in a vacuum desiccator over phosphorus pentoxide.

Compound JF 36

25.3 g (0.1 mol) of 6-(2-carboxybenzoyl)-N-ethyl-1,2,3,4-tetrahydroquinoline and 20.1 (0.1 mol) of N-ethyl-7-isopropenyl-1,2,3,4-tetrahydroquinoline are dissolved in 500 ml of dichloromethane and treated with 60 g of phosphorus pentoxide. The mixture is heated under reflux for 2 h, allowed to cool and the solvent is distilled off in vacuo. The residue is treated with conc. sulfuric acid. This solution is stirred at room temperature for 30 min. After this, the sulfuric acid solution is added to 1 000 ml of ice-cooled ethanol and treated dropwise with 50 ml of 60% strength perchloric acid and 5 l. The dye precipitated is filtered off and dried over phosphorus pentoxide in a vacuum desiccator.

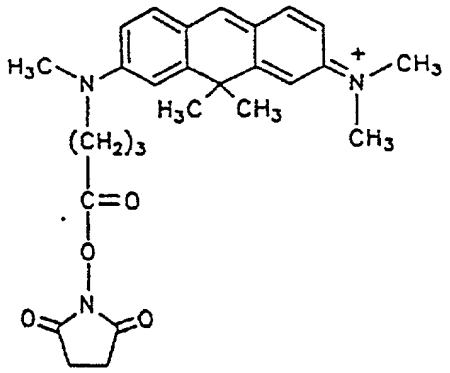
Compound JF 37

39.1 g (0.1 mol) of 6-(2-carboxy-3,4,5,6-tetrachlorobenzoyl)-N-ethyl-1,2,3,4-tetrahydroquinoline and 20.1 (0.1 mol) of N-ethyl-7-isopropenyl-1,2,3,4-tetrahydroquinoline are dissolved in 500 ml of dichloromethane and treated with 60 g of phosphorus pentoxide. The mixture is heated under reflux for 2 h, allowed to cool and the solvent is distilled off in vacuo. The residue is treated with conc. sulfuric acid. This solution is stirred at room temperature for 30 min. After this, the sulfuric acid solution is added to 1 000 ml of ice-cooled ethanol and treated dropwise with 50 ml of 60% strength perchloric acid and 5 l. The dye precipitated is filtered off and dried over phosphorus pentoxide in a vacuum desiccator.

C. Examples of conjugate formation

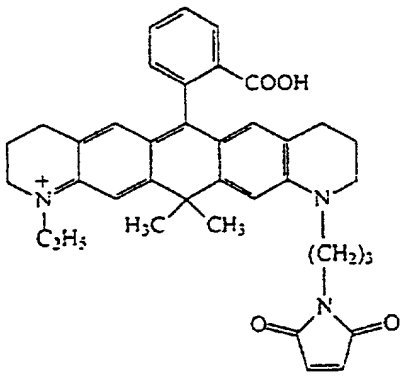
JA 262 active ester

0.1 mmol of JA 262 is dissolved in 20 ml of acetonitrile with 0.2 mmol of N-hydroxysuccinimide and 0.2 mmol of dicyclohexylcarbodiimide. The solution is stirred at room temperature for 4 h and the product mixture is concentrated on a rotary evaporator. Purification is carried out by chromatography (HPLC, RP 18).



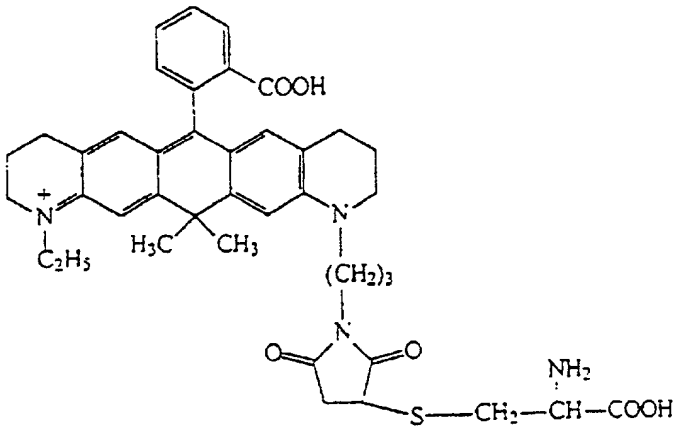
JF 43 maleimide

100 mg of JF 43 (0.16 mmol) are dissolved in 10 ml of dried DMSO and treated with 100 mg (1 mmol) of maleic anhydride. The solution is stirred at room temperature for 24 h. 50 ml of 10% strength aqueous sodium perchlorate solution are added dropwise and the solid precipitated is filtered off. The solid is suspended in 5 ml of acetic anhydride with 25 mg of sodium acetate and heated to 80°C for 30 min. The mixture is cooled and 30 ml of 10% strength aqueous sodium perchlorate solution are added dropwise. The solid is filtered off and dried.



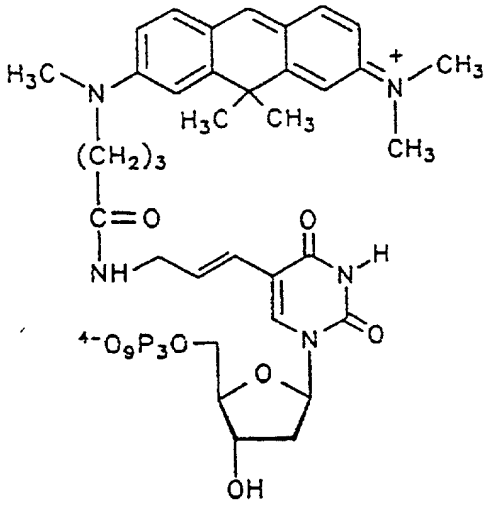
JF 43-cysteine conjugate

70 mg (0.1 mmol) of JF 43 maleimide are dissolved in 20 ml of ethanol and treated in portions with 12 mg (0.1 mmol) of cysteine. The solution is stirred at room temperature for 30 min. After this, 50 ml of 10% strength aqueous sodium perchlorate solution are added dropwise and the solid precipitated is filtered off and dried.



JA 262-dUTP conjugate

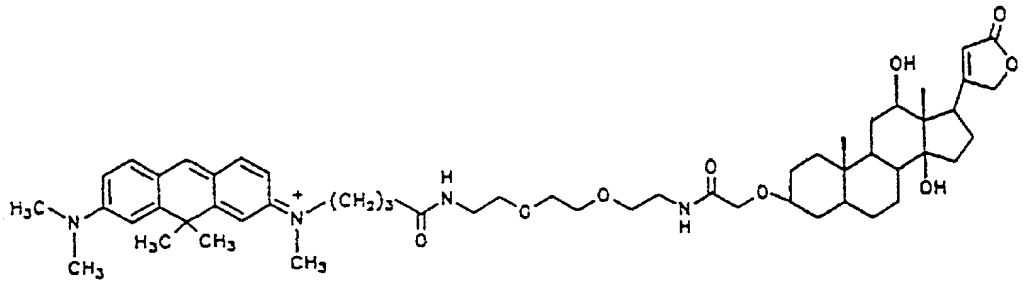
10 µmol of 5-(3-aminoallyl)-dUTP are dissolved in 0.5 ml of 0.1 M sodium borate buffer (pH 8) and treated with a solution of 5 µmol of JA 262 active ester in 1 ml of amine-free dimethylformamide. The solution is stirred at room temperature for 15 h. The solvents are distilled off in vacuo and the residue is purified by chromatography (RP 18).



JA 262-digoxin-3-carboxymethyl ether-diaminodioxaoctane conjugate (Dig-CME-DADOO)

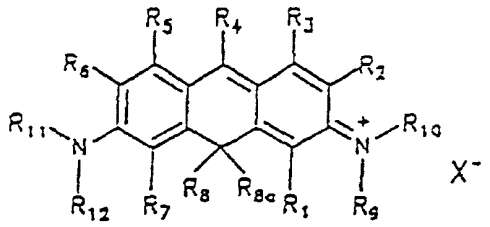
0.02 mmol of JA 262 active ester are stirred in acetonitrile at room temperature for 18 h with 0.02 mmol of Dig-CME-DADOO. The solvent is distilled

off and the residue is purified by chromatography.



Claims

1. The use of compounds of the general formula I



I

as labeling groups in a procedure for the detection of an analyte, where

R_1, R_2, R_3, R_4, R_5, R_6 and R_7 are in each case independently hydrogen, halogen, a hydroxyl, amino, sulfo or carboxyl or aldehyde group or a saturated or unsaturated, straight-chain, branched or cyclic hydrocarbon group having up to 20 C atoms, where the hydrocarbon groups include alkyl, alkenyl, alkynyl, cycloalkyl, aryl, in particular phenyl, or/and heteroaryl radicals and optionally heteroatoms such as oxygen, sulfur or nitrogen atoms or/and two or more substituents, preferably selected from halogens, hydroxyl, amino, sulfo, phospho, carboxyl, aldehyde, C_1-C_4-alkoxy or/and C_1-C_4-alkoxycarbonyl groups,

or one or more of the radicals R_1-R_7, in each case with adjacent substituents, form a ring system which can contain one or more multiple bonds,

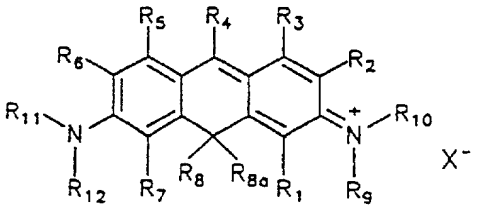
R_8 and R_{8a} in each case independently are a saturated or unsaturated, straight-chain, branched or cyclic hydrocarbon group having up to 20 carbon atoms, e.g. a C_1-C_6-alkyl group, in particular methyl, ethyl, propyl or/and butyl, or an aryl or heteroaryl group, in particular phenyl, which optionally contain heteroatoms such as oxygen, sulfur or nitrogen atoms or/and one or more substituents, preferably selected from halogens, hydroxyl, amino, sulfo, phospho, carboxyl,

aldehyde, C_1-C_4-alkoxy or/and C_1-C_4-alkoxycarbonyl groups,
or alternatively R_8 and R_{8a} can form a ring system,
R_9, R_{10}, R_{11} and R_{12} in each case independently are hydrogen or a saturated or unsaturated, straight-chain, branched or cyclic hydrocarbon group having up to 20 C atoms, e.g. polyether, phenyl, phenyl-alkyl having 1-3 C atoms in the chain, where the hydrocarbon groups can optionally contain hetero-atoms such as oxygen, sulfur or nitrogen atoms or/and one or more substituents, preferably selected from halogens, hydroxyl, amino, sulfo, phospho, carboxyl, carbonyl, alkoxy or/and alkoxy-carbonyl groups,
or one or more of the radicals R_9-R_{12}, in each case with adjacent substituents, form a ring system which can contain one or more multiple bonds,
where $-N(R_9)(R_{10})$ or/and $=N(R_{11})(R_{12})$ can be replaced by $-OR^9$ or/and $=O$,
and X is optionally anions present for charge equalization.

2. The use as claimed in claim 1,
characterized in that
the compound I is coupled covalently to a receptor specific for the analyte to be detected.

3. The use as claimed in claim 1 or 2,
characterized in that
the detection procedure is selected from nucleic acid hybridization procedures and immunochemical procedures.

4. A compound of the general formula I



I

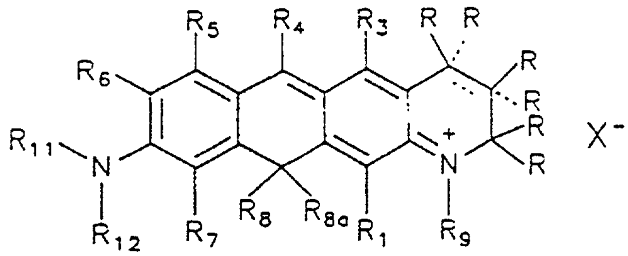
where R_1-R_{12} and X have the meanings indicated in claim 1, with the proviso that if R_1-R_3 and R_5-R_7 are hydrogen and R_8, R_{8a} and R_9-R_{12} are methyl, R_4 is not hydrogen, methyl, isopropyl, phenyl, 2,6-dimethylphenyl or 2-isopropenylphenyl.

5. A compound as claimed in claim 4, **characterized in that** R_6 is bridged with R_{11} or/and R_7 with R_{12}, R_1 with R_{10} or/and R_2 with R_9 and form a ring system which preferably contains 5- or 6-membered rings which can contain one or more multiple bonds.

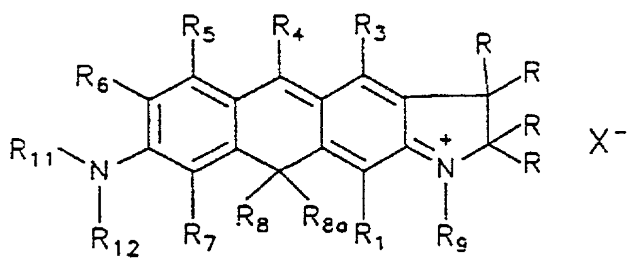
6. A compound as claimed in claim 4 or 5, **characterized in that** R_4 is hydrogen, C_1-C_6-alkyl or a radical containing an aromatic ring system.

7. A compound as claimed in one of claims 4 to 6, **characterized in that** R_8 and R_{8a} are in each case independently methyl, ethyl or/and phenyl.

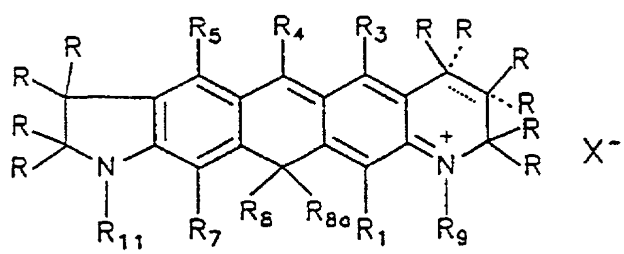
8. A compound as claimed in one of claims 4 to 6, which corresponds to one of the general formulae IVa to IVe.



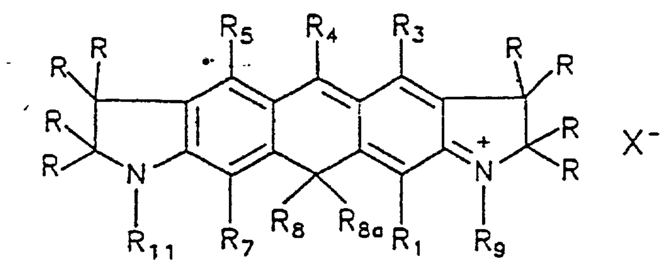
IVa



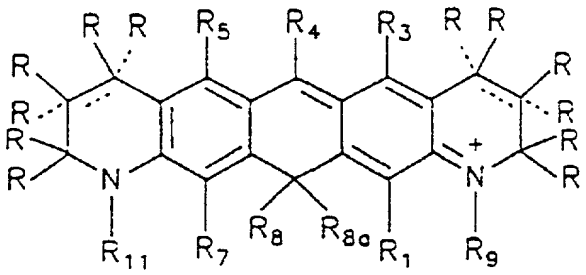
IVb



IVc



IVd



IVe

in which
the dashed lines are optionally double bonds, and in the presence of the double bonds the radicals R bonded via a dashed line are absent,
R_1, R_3, R_4, R_5, R_6, R_7, R_8, R_{8a}, R_9, R_{11}, R_{12} and X are as defined in claim 1,
and R, on each occurrence, can be identical or different and is defined as R_1-R_7 in claim 1.

9. A compound as claimed in one of claims 4 to 8, **characterized in that** it has a group capable of covalent coupling.

10. A compound as claimed in claim 9, **characterized in that** the coupling group is -COOH, $-NH_2$, -OH or/and -SH.

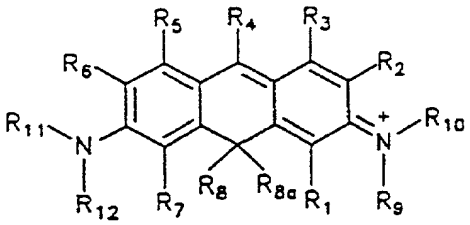
11. A compound as claimed in claim 9 or 10, **characterized in that** it is coupled to a carrier or/and to a biomolecule via coupling groups.

12. A compound as claimed in claim 10, **characterized in that** the carrier is selected from porous glass, ion-exchange resins, dextrans, cellulose, cellulose derivatives or/and hydrophilic polymers.

13. A compound as claimed in claim 10, **characterized in that** the biomolecule is selected from peptides, poly-

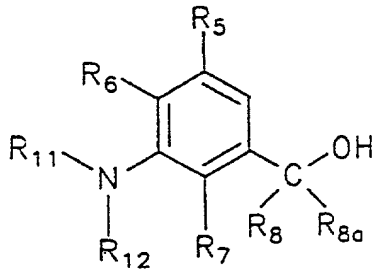
peptides, nucleotides, nucleosides, nucleic acids, nucleic acid analogs or/and haptens.

14. A process for the preparation of compounds of the general formula I



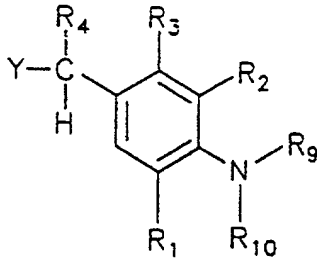
I

where R_1-R_{12} and X have the meanings indicated in claim 1,
characterized in that
a compound of the general formula II



II

in which R_5, R_6, R_7, R_8, R_{8a}, R_{11}, R_{12} are as defined in claim 1, or the dehydration product of II is reacted with a compound of the general formula III or its dehydration product



III

in which R_1-R_4, R_9 and R_{10} are as defined in claim 1 and Y is a halogen, in particular bromine, a

hydroxyl or thio group, in a suitable solvent, under acidic conditions and in the presence of a catalyst and the compound formed by ring closure between the compound II or its dehydration product and the compound III or its dehydration product is optionally reacted by oxidation to give the dye I.

15. The process as claimed in claim 14, **characterized in that** the solvent is a nonpolar solvent, in particular methylene chloride, 1,2-dichloroethane or chloroform.

16. The process as claimed in one of claims 14 to 15, **characterized in that** the catalyst is boron trichloride.

17. The process as claimed in one of claims 14 to 16, **characterized in that** the acid is sulfuric acid, phosphoric acid or polyphosphoric acid.

18. The process as claimed in one of claims 14 to 17, **characterized in that** the oxidant is tetrabutylammonium (meta)periodate.

19. The process as claimed in one of claims 14 to 18, **characterized in that** the compound (I) is obtained without isolation of intermediates.

Absorption and fluorescence spectra in ethanol

Fig. 1: AZ 2

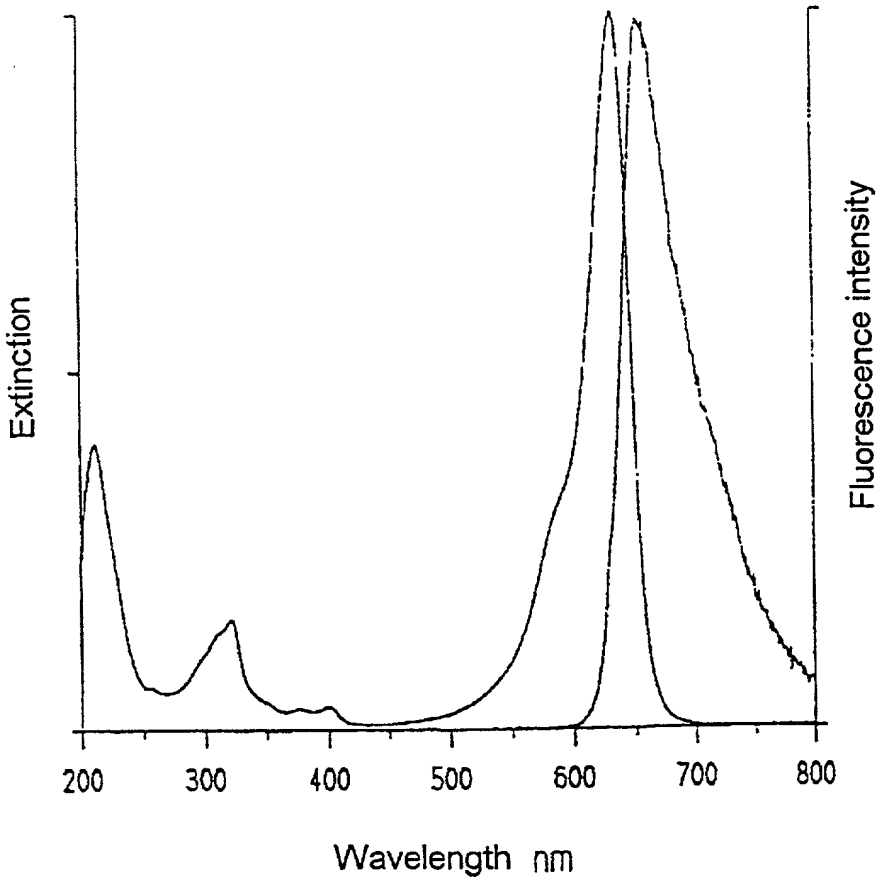


Fig. 2: AZ 13

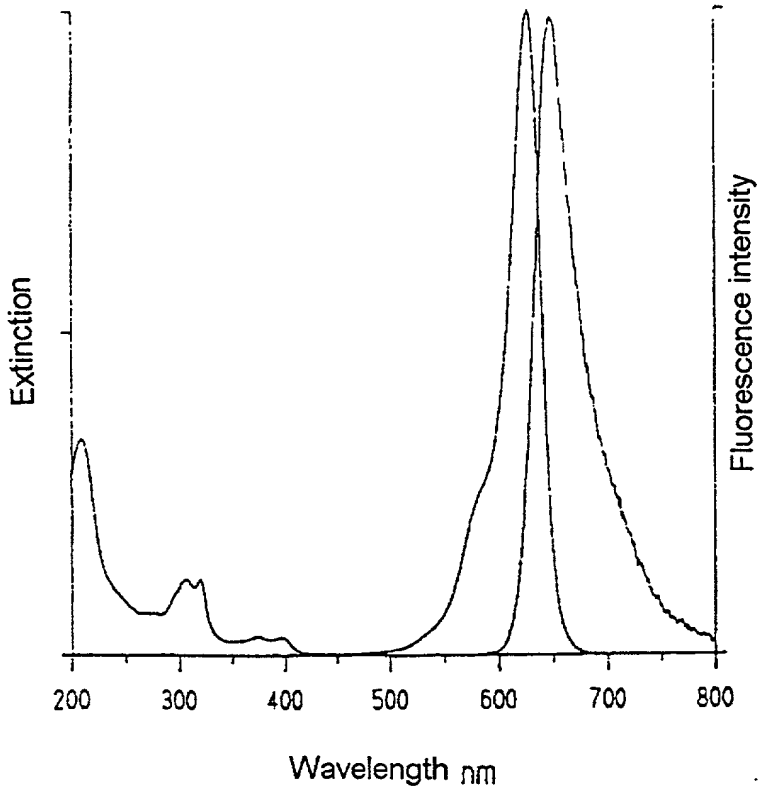


Fig. 3: JA 268

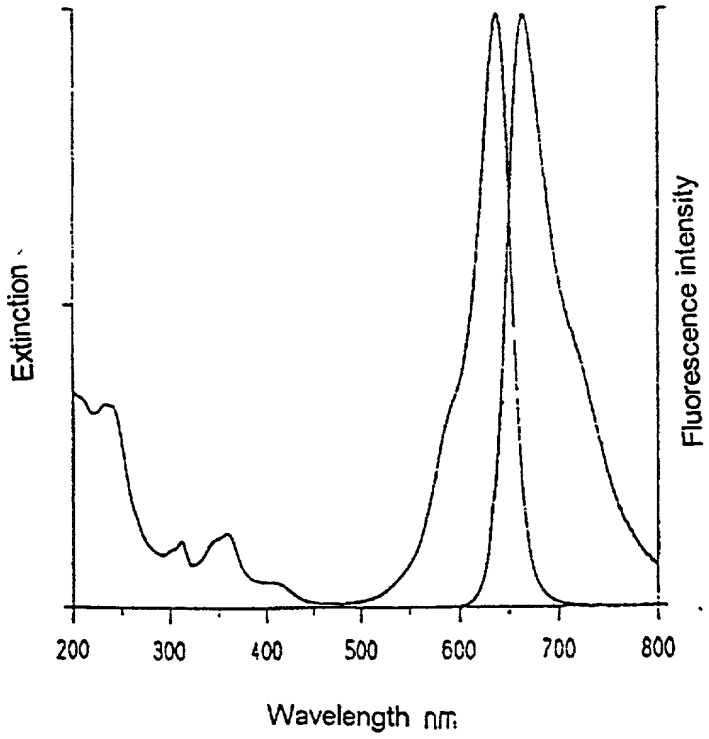
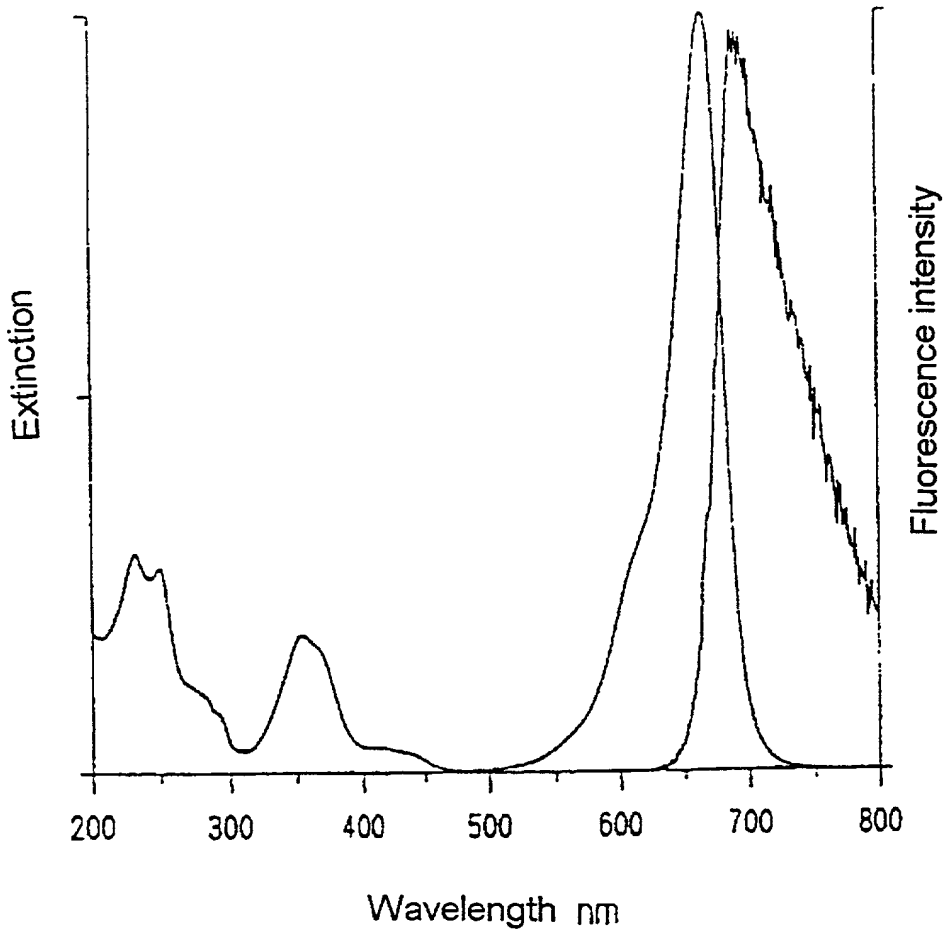


Fig. 4: AZ 11



Declaration and Power of Attorney for Patent Application
Erklärung für Patentanmeldungen mit Vollmacht
German Language Declaration

Als nachstehend benannter Erfinder erkläre ich hiermit an Eides Statt:

daß mein Wohnsitz, meine Postanschrift, und meine Staatsangehörigkeit den im Nachstehenden nach meinem Namen aufgeführten Angaben entsprechen,

daß ich, nach bestem Wissen, der ursprüngliche, erste und alleinige Erfinder (falls nachstehend nur ein Name angegeben ist) oder ein ursprünglicher, erster und Miterfinder (falls nachstehend mehrere Namen aufgeführt sind) des Gegenstandes bin, für den dieser Antrag gestellt wird und für den ein Patent beantragt wird für die Erfindung mit dem Titel:

NEUE CARBOPYRONIN-FLUORESZENZ-FARBSTOFFE

deren Beschreibung
(zutreffendes ankreuzen)

[ ] hier beigefügt ist.
[X] wurde angemeldet am 19. April 2000 unter der U.S.-Anmeldungs Nr. oder unter der Internationalen Anmeldenummer im Rahmen des Vertrags über die Zusammenarbeit auf dem Gebiet des Patentwesens (PCT) PCT/EP00/03568 und am _____ abgeändert (falls zutreffend).

Ich bestätige hiermit, daß ich den Inhalt der obigen Patentanmeldung einschliesslich der Ansprüche durchgesehen und verstanden habe, die eventuell duch einen Zusatzantrag, wie oben erwähnt, abgeändert wurde.

Ich erkenne meine Pflicht zur Offenbarung irgendwelcher Informationen an, die für die Prüfung der vorliegenden Anmeldung in Einklang mit Titel 37, Code of Federal Regulations, §1.56 von Belang sind.

Ich beanspruche hiermit ausländische Prioritätsvorteile gemäss Titel 35, US-Code, §119(a)-(d), bzw. §365(b) aller unten angegebenen Auslandsanmeldungen für ein Patent oder Erfinderurkunden, oder §365(a) aller PCT internationalen Anmeldungen, welche wenigstens ein Land ausser den Vereinigten Staaten von Amerika benennen, und habe nachstehend durch ankreuzen sämtliche Auslandsanmeldungen für Patente oder Erfinderurkunden oder PCT internationale Anmeldungen angegeben, deren Anmeldetag dem der Anmeldung, für welche Priorität beansprucht wird, vorangeht.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL CARBOPYRONINE FLUORESCENCE DYES

the specification of which
(check one)

[ ] is attached hereto
[X] was filed on 19 April 2000 as United States Application Number or PCT International Application Number PCT/EP00/03568 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Applications (Frühere ausländische Anmeldungen)			Priority Claimed? Priorität beansprucht?	
199 19 119.0 (Number) (Nummer)	Germany (Country) (Land)	27/April/1999 (Day/Month/Year Filed) (Tag/Monat/Jahr eingereicht)	[X] Yes Ja	[ ] No Nein
_____ (Number) (Nummer)	_____ (Country) (Land)	_____ (Day/Month/Year Filed) (Tag/Monat/Jahr eingereicht)	[ ] Yes Ja	[ ] No Nein

Ich beanspruche hiermit gemäss Titel 35, US-Code, §119(e), den Vorzug aller unten aufgeführten US-Hilfsanmeldungen

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) below

_____ (Application No. / Anmeldenr.) _____ (Filing Date / Anmeldedatum)

_____ (Application No. / Anmeldenr.) _____ (Filing Date / Anmeldedatum)

Ich beanspruche hiermit gemäss Titel 35, US-Code, §120, den Vorzug aller unten aufgeführten US-Patentanmeldungen bzw. §365(c) aller PCT internationalen Anmeldungen, welche die Vereinigten Staaten von Amerika benennen, und erkenne, insofern der Gegenstand eines jeden früheren Anspruchs dieser Patentanmeldung, bzw. PCT internationalen Anmeldung in einer gemäß dem ersten Absatz von Titel 35, US-Code §112 vorgeschriebenen Art und Weise offenbart wurde, meine Pflicht zur Offenbarung jeglicher Informationen an, die zur Prüfung der Patentfähigkeit in Einklang mit Titel 37, Code of Federal Regulations, §1.56 von Belang sind und im Zeitraum zwischen dem Anmeldedatum der früheren Patentanmeldung und dem nationalen oder im Rahmen des Vertrags über die Zusammenarbeit auf dem Gebiet des Patentwesens (PCT) gültigen internationalen Anmeldedatum bekannt geworden sind.

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(Appl. No.) (Anmeldenr.)	(Filing Date) (Anmeldedatum)	(Status) (patentiert, anhängig aufgegeben)	(Status) (patented, pending abandoned)
(Appl. No.) (Anmeldenr.)	(Filing Date) (Anmeldedatum)	(Status) (patentiert, anhängig aufgegeben)	(Status) (patented, pending abandoned)

Ich erkläre hiermit, daß alle von mir in der vorliegenden Erklärung gemachten Angaben nach meinem besten Wissen und Gewissen der vollen Wahrheit entsprechen, und daß ich diese eidesstattliche Erklärung in Kenntnis dessen abgebe, daß wissentlich und vorsätzlich falsche Angaben gemäss §. 1001, Titel 18 US-Code strafbar sind und mit Geldstrafe und/oder Gefängnis bestraft werden können, und daß derartig wissentlich und vorsätzlich falsche Angaben die Rechtswirksamkeit der vorliegenden Patentanmeldung oder eines darauf erteilten Patentes gefährden können.

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

HENRY M. FEIEREISEN
Reg. No. 31,084

URSULA B. DAY
Reg. No. 47,296

Telefongespräche bitte richten an:
(Name und Telefonnummer)

Direct telephone calls to:
(Name and telephone number)

HENRY M. FEIEREISEN
(212) 244-5500

Postanschrift:

Send Correspondence to:

CUSTOMER NO. 020151

Voller Name des einzigen oder ursprünglichen Erfinders

Full name of first inventor

Karl-Heinz Drexhage

Unterschrift des Erfinders Datum

Inventor's Signature Date

Karlheinz Drexhage 17 October 2001

Wohnsitz

Residence

Siegen/Germany DEX

Staatsangehörigkeit

Citizenship

Germany

Postanschrift

Post Office Address

Schanzenweg 50
57076 Siegen/Germany

Voller Name des zweiten Erfinders

Full name of second inventor

Jutta Arden-Jacob

Unterschrift des Erfinders Datum

Inventor's Signature Date

Jutta Arde-Jacob 17 October 2001

Wohnsitz

Residence

Zirndorf/Germany DEX

Staatsangehörigkeit

Citizenship

Germany

Postanschrift

Post Office Address

Am Hügel 25
90513 Zirndorf/Germany

Voller Name des dritten Erfinders — Full name of third inventor

Jörg Frantzeskos

Unterschrift des Erfinders — Datum — Inventor's Signature — Date

Frantzeskos 20.10.2001

Wohnsitz — Residence

Wenden/Germany DEX

Staatsangehörigkeit — Citizenship

Germany

Postanschrift — Post Office Address

Hauptstraße 44
57482 Wenden/Germany

Voller Name des vierten Erfinders — Full name of ~~third~~ fourth inventor

ALEXANDER ZILLES

Unterschrift des Erfinders — Datum — Inventor's Signature — Date

A. [signature] × 25.10.01 ×

Wohnsitz — Residence

West Yorkshire, Leeds LS7 3ED / Great Britain GB3

Staatsangehörigkeit — Citizenship

Germany

Postanschrift — Post Office Address

16 Methly Terrace
West Yorkshire, Leeds LS7 3ED
Great Britain